

The University of Chicago

**CYTOLOGICAL STUDIES OF
THE DECIDUAL REACTION IN THE RAT
DURING EARLY PREGNANCY AND
IN THE PRODUCTION OF
*DECIDUOMATA***

AN EXPANSION OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE BIOLOGI-
CAL SCIENCES IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1935

By

ROBERT HENRY KREHBIEL

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CYTOLOGICAL STUDIES OF THE DECIDUAL REACTION IN THE RAT DURING EARLY PREGNANCY AND IN THE PRODUCTION OF DECIDUOMATA¹

(Sixteen figures)

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IMPLANTATION

THE greater part of our knowledge concerning the decidual cells formed in the antimesometrial wall of rodent uteri during early pregnancy has been obtained from observations of that organ in its relation to the attachment and growth of the embryo. Since Tafani (1886) and Duval (1891) noted this reaction on the part of the mouse and rat uterus, numerous investigations have been added to the published material dealing with the subject. However, very few of these publications deal with the uterine mucous membrane except in so far as it aids in the explanation of the embryonic activity. The histological value of the work of Spee (1901) on the guinea pig and Burckhard (1901) on the mouse is well known. Jenkinson (1902), concerned chiefly with the fetal giant cells, gave a sketchy account of the glycogen and lipoid content of the mouse uterus. From these and other studies we know that in many rodents an antimesometrial decidua develops.

L. Loeb (1907) discovered that the antimesometrial wall of the guinea-pig uterus produces decidual tissue after mechanical injury. This has been abundantly verified. Although he recognized that the experimentally produced enlargements resembled decidua, he regarded them as tumors and called them "deciduomata." Unfortunately, the term, with all its implications, has been established by general usage.

Lataste (1887), in his observations of the breeding habits of rodents, noted that they would mate following parturition and that: "Ches les Muridés, quand la femelle entre en lactation au début de sa grossesse, la durée de la gestation est de trois périodes génitales; dans tous les autres cas, elle n'est que deux périodes." His *périodes génitales* were 10-11 days long. In explanation of this he postulated: "Les ovules après fécondation, ont leur développement arrête pendant la première des trois périodes génitales de cette gestation." Duval (1891) provided evidence of the same in the rat. Kirkham (1916) and Enzmann, Saphir, and Pincus (1932) have confirmed Lataste's postulation by showing that the delay is due to the failure of the embryo to become attached at the proper time—a phenomenon which when explained, I believe, will certainly find many if not all its basic factors in the state or condition in which the uterine mucous membrane is at that time.

The explanation of the various interpretations may be found in the lack of an ade-

¹ I wish to thank Dr. G. W. Bartelmez for his constructive criticism during the course of the work and for his continued encouragement. The drawings are the work of Miss Agnes Mixon.

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quate anatomical knowledge of this tissue. Consequently, the problem has been approached from a cytological viewpoint in an endeavor to provide in part an anatomical foundation which may be used in the correlation of the various factors involved.

TABLE 1

RAT No.	GESTATION AGE		RAT No.	GESTATION AGE	
	Day	Hour		Day	Hour
1	7	23	46	7	22
2	7	12	47	11	1
3	6	15	49*	9	12
4	6	15	50	10	23
5	6	18	51†	6	11
7	7	23	52†	7	12
8	7		53†	11	
11	8	21	54*	9	
12	6		57†	8	20
15	7		58†	8	12
16	7	18	61†	6	6
19	7	12	62†	6	2
20	6	23	63†	6	8
25	10	12	64†	6	11
26	5	12	65†	6	2
27	9	11	67†	7	23
28	7	22	68†	6	15
29	8	20	69†	7	13
30	7	8	70†	7	
31	10	12	72†	6	18
33	10		73†	6	21
35	8	12	77†	8	12
36	7	3	79*	9	22
37	6	2	80*	8	13
38	6	6	81†	12	1
39	9	18	82†	6	19
40	8	5	83†	10	15
41	12		85†	10	12
44	6	12	86†	6	14
45	6	4			

* Denotes animals in which deciduomata were produced in 1 (pregnant) horn.

† Denotes animals in which 1 horn was kept empty for deciduoma studies by tying off and removing part of the Fallopian tube.

In bilateral pregnancies the number of embryos varies from 3 to 13 with an average of 7.2 embryos per litter.

MATERIAL AND METHODS

Gestational ages were dated from the time of ejaculation. The use of this method introduces no significant error, since the variations noted between embryos of the same copulation age, obtained from different animals, were seldom greater than those found in a single horn. Table 1 gives the number of animals used and the gestation age of each.

The animals were usually killed by a blow over the head, the uterus removed, and the loci of implantation fixed in various fluids while the animal was still alive or very few minutes after death. A few vascular profusions of the fixative were done. In order to

determine the vascular pattern of the decidua, an 8 per cent solution of colloidal mercuric sulphide was injected into the internal jugular vein while the animal was under ether, the uterine blood vessels were clamped off, and the organ removed to the fixing fluid (Daron, 1936).

Numerous fixations were used with varying degrees of success. The most valuable proved to be Bensley's modification of Zenker (2.5 per cent bichromate, 5 per cent mercuric chloride, and 10 per cent formol) followed by a 3-5 per cent potassium bichromate mordant. This fixative not only preserved the general histological structures but also proved most adequate in the preservation of lipoids and mitochondria. Regaud and a 10 per cent formalin solution were also used in lipid preparations by the Sudan III method. The former is good, whereas formalin introduces difficulties in sectioning the material. Müller's fluid and Maximow's formol-osmic Zenker were used in osmic methods for lipid.

Romeis' (1929) solution of 40 per cent Sudan III, followed by Erlich's haematoxylin, was used in staining lipoids in frozen sections. With a few alterations of Zwemer's (1933) method, sections from 3 to 8 μ in thickness were obtained. The sections were removed from the knife and placed upon a clean slide. Sufficient water to straighten the sections was added, and the whole allowed to dry at room temperature. This leaves a thin film of gelatin over the tissue, which preserves it as well as paraffin and may be soaked off in water without the loss of sections. If too much water is added, the tissue is not covered by gelatin and cracks, whereas if not enough is used, too much gelatin remains, and the sections are likely to fall off. Zwemer's suggestion of using formalin proved disadvantageous because the formalin leaves the gelatin insoluble in water, thus masking the staining of the material. Sections thus obtained may also be stained by other methods, especially for the sharp differential staining of mitochondria.

In addition to the formol Zenker solution, Maximow's fluid, followed by 3-5 days' osmication and subsequent bleaching in potassium permanganate, was also used in the demonstration of mitochondria which were stained with aniline acid fuchsin followed by Mallory's aniline blue stain. Fresh tissues, sometimes stained supravitaly with janus-green B for mitochondria, were studied as a control of the fixed material.

One part formol to nine parts absolute alcohol (O'Leary) was used in fixing glycogen which was stained with Best's carmine. Saliva-digested controls were made in each case. In some specimens the iodine reaction was also used. Weaker alcohol solutions failed to preserve all the glycogen. Good staining of chromidial substance with toluidin blue was obtained after absolute alcohol formol fixation. Reticulum fibers were demonstrated by the Bielchowsky-Foote silver method after formol Zenker fixation.

OBSERVATIONS

A review of the histology of implantation is necessary to clarify the nomenclature to be used. A general survey of the process is presented in Figure 1, which diagrammatically shows the antimesometrial and mesometrial decidual formations which differ in character and in time of appearance.

EPITHELIAL CHANGES

During the fifth day, before implantation, the uterine epithelium is uniform throughout. With the start of the sixth day, a series of transformations in the uterine surface epithelium occurs which divides it into four areas: (1) the *antimesometrial area*, which becomes pseudostratified; (2) the *implantation area*, where the epithelium surrounding the embryo flattens to a low cuboidal or squamous type; (3) the *transitional area*—ap-

proximately half of the epithelium lying mesometrial to the embryo shows a transition from the high columnar epithelium of fifth-day cases to the low cuboidal found in the implantation area; and (4) the tall columnar *mesometrial area*.

With this differentiation the diameter of the implantation chamber has become greater than that of the remainder of the lumen. With the continued growth of the embryo the implantation area is further stretched. The first replacement of this epithelium is brought about by the migration of a single trophoblastic cell between the flattened epithelial cells none of which is destroyed at first. This trophoblastic migration continues until at the end of the sixth day the trophoblast is in contact with the stroma throughout the implantation area. At that time the base of the embryo, the ob-placental pole, lies on an antimesometrial epithelial shelf, the sides are in contact with the stroma, and the Träger touches the lowermost cells of the transitional area. It is to be noted that the epithelium forms a blunt wedge between the embryonic and the maternal tissue at the antimesometrial and mesometrial edges of the denuded area.

During the seventh day the antimesometrial area undergoes a fatty degeneration which is completed some time during the eighth day (cf. p. 220). While this is going on, the lumen continues to lengthen along the implantation axis (cf. p. 218), whereas the epithelial cells do not multiply. Thus, more and more surface is to be covered, and the epithelial cells of the transitional area become lower; their nuclei become first spherical and then flattened.

Until the beginning of the eighth day the alterations in the transitional area are merely changes in cellular form. However, at this time there has been some extravasation of blood into the lumen. The blood lifts the lowermost portion of the transitional epithelium from its basement membrane, and fatty degeneration ensues. By the middle of the ninth day it is gone. It is not until late in the tenth day that the epithelium becomes continuous antimesometrially to the implantation site.

The high columnar cells of the mesometrial area rest upon a reticular basement membrane and have definite cell outlines and terminal bars. The oval nuclei are basal. The cytoplasm is abundantly supplied with spheroidal, rodlike, and filamentous mitochondria with a predominance of the latter. In 4μ sections they fill the distal end of the cell, making it difficult to distinguish the individual mitochondria. At the sides and basal to the nucleus they tend to be longer and thicker filaments lying parallel to the long axis of the cell. There is no change in the mitochondria when differentiation in cellular form occurs in the various areas. Normal-appearing mitochondria may be found in cells lying next to the migratory trophoblastic ectoderm. Since these uterine cells are also normal in other respects, there is no evidence of injury during the early phase of invasion. During the seventh day the increasing amount of lipoid in the cells of the antimesometrial area makes it impossible to demonstrate normal mitochondria, if any are present. The same is true of the greater part of the transitional area after the eighth day.

STROMA CHANGES

Just before embryonic attachment occurs, early in the sixth day, the changes in the stroma become localized and orientated about the implantation chamber. Thus, a line drawn through the mesometrium and the center of the embryo passes through the central axis of decidual activity and may be called the "implantation axis." These changes consist of an enlargement of the subepithelial cells into decidual cells, a differentiation of the capillary bed, and a localization of glands which enable one to distinguish an antimesometrial and a mesometrial region (Fig. 1, *A* and *B*).

The enlargement of the subepithelial cells begins late in the fourth day, and by the time the embryo has reached its definitive position (last half of fifth day) the "primary

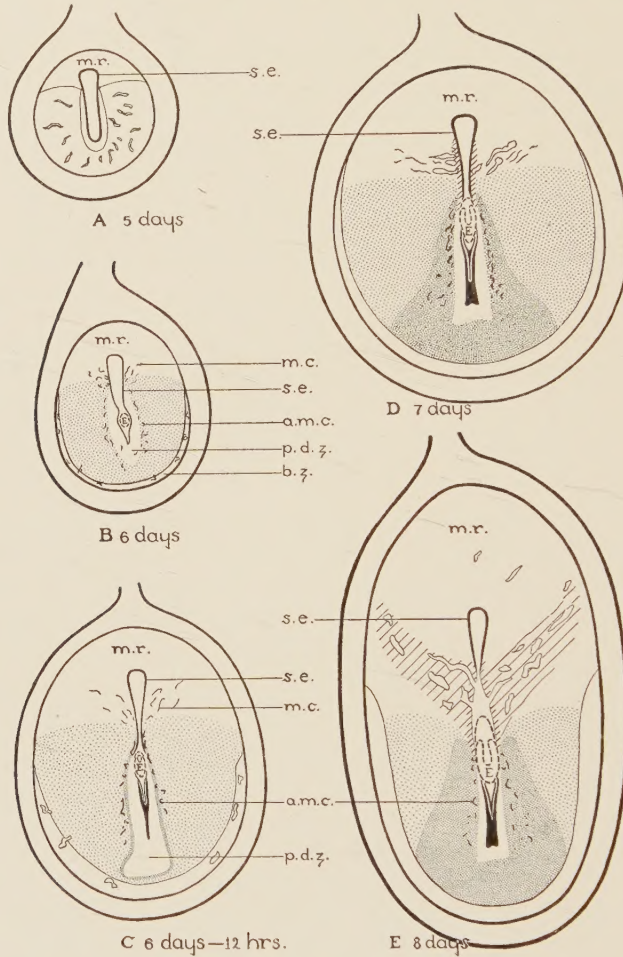


FIG. 1.—A series of diagrammatic cross-sections to show the differentiation and development of the various regions of the decidua: *A*, 5 days; *B*, 6 days; *C*, 6½ days; *D*, 7 days; *E*, 8 days; *F*, 9 days; *G*, 10 days; and *H*, 11 days. It is to be noted that the growth of the antimesometrial (lipoid) region consists of two phases: (1) an early, but brief, development about the implantation axis differentiates the primary decidua and implantation zones; and (2) a second phase of growth differentiates the secondary decidua zone which ultimately forms the decidua capsularis. The development of the mesometrial (glycogenic) region progresses rapidly late in the seventh day and soon becomes the dominating feature, giving rise to the maternal placenta. Embryonic structures (*E*) are represented by broken lines. $\times 13$.

decidua zone" (Fig. 1, *B* and *C*, *pdz*) is differentiated, and only small intercellular spaces and reticulum separate the closely packed cells. The primary decidua zone extends in the implantation axis for varying distances antimesometrially.

The primary decidual zone is comparatively free from prominent capillaries, and as it spreads they gradually recede from the lumen. In early sixth-day cases a narrow mesh-

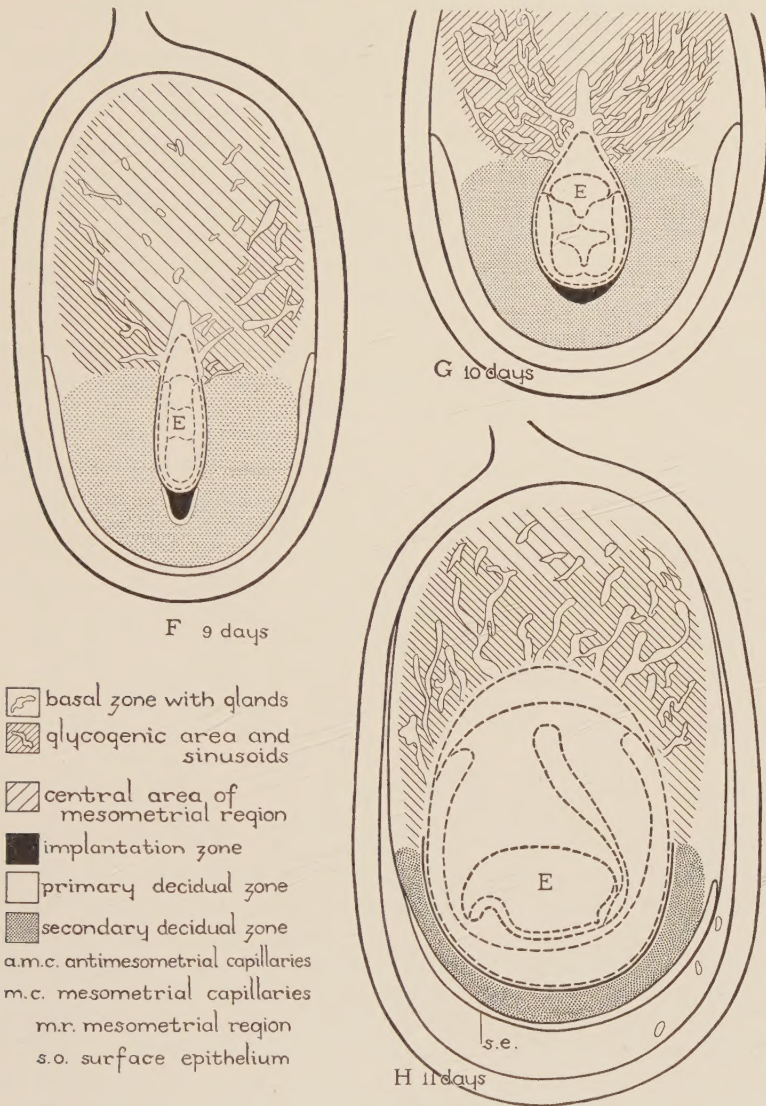


FIG. 1.—Continued

work of capillaries surrounding the primary decidual zone becomes pronounced. At the mesometrial extremity of the zone the capillaries extend to the epithelium (Fig. 1, B, *amc*), and here their radial arrangement accentuates the differentiation of mesometrial and antimesometrial regions, which are further differentiated by the presence of glands in

the antimesometrium. Between the glands are loosely associated stellate stroma cells and a heavy reticulum, which stains metachromatically with toluidin blue. During the last half of the fifth day and the first few hours of the sixth, the stroma surrounding the primary decidual zone becomes highly oedematous, and the glands are pushed to the sides of the enlarging area. Thus, in cross-sections through an implantation site they are found next to the antimesometrial musculature in a narrow strip of inactive stroma which may be called the "basal zone" (Fig. 1, *B*).

During the first half of the sixth day the development of the antimesometrial region results in a localized thickening of the uterine horn. The rapidly differentiating primary zone spreads to the basal zone. However, the cells at the core of the decidual zone lag in their development and stain more intensely, thus differentiating an additional zone which may be called the "implantation zone." This is indicated in solid black in Figure 1, *C*. The reticulum of this zone is radially organized, while in the larger-celled primary zone its meshes are irregular and wide. However, the fibers in both zones have lost their metachromatic reaction to toluidin blue—a characteristic feature of the decidual zones as they increase in extent. The capillary meshwork at the sides of the primary zone retains its position as the latter enlarges during the sixth day (Fig. 1, *C*, *amc*).

Before the middle of the sixth day a new decidual development appears at the periphery of the primary zone and is characterized by its lipid-laden decidual cells. This secondary decidual zone at first undergoes an extensive hyperplasia, and many binucleated cells appear. Hypertrophy continues through the last of the seventh and eighth days to reach a climax during the ninth day and clearly differentiates the antimesometrial region (Fig. 1, *F*, stipple). The formation of binucleated cells throughout the secondary decidual zone is a feature, presumably, of the rapid hyperplasia since no indication of amitosis or cell fusion has been observed. Two nuclei, but never any more, may be present in small as well as in large decidual cells of this zone.

The changes occurring in the primary zone are definitely associated with the enlargement of the implantation chamber. As it increases in size, its pressure and that of the growing tissue of the secondary zone cause the primary zone to narrow, as its cells which border the implantation chamber flatten (Fig. 1, *E*). Some cells are degenerating and have pyknotic nuclei and a fibrous cytoplasm. With this narrowing the capillaries reach the surface of the implantation chamber at the beginning of the eighth day and are open so that extravasation into the lumen occurs. In this connection it is to be noted that fetal giant cells lie against maternal tissue and at the open mouths of the capillaries.

The loss of tissue along the sides of the implantation chamber continues but soon becomes secondary to the degenerative process in the implantation zone. This zone, as well as the primary zone, shortens during the rapid growth of the secondary zone. Many of its cells, especially those which have replaced the antimesometrial epithelium during the eighth day, undergo a fatty degeneration, and the others become fibrous. By the middle of the ninth day numerous intact capillaries appear in the implantation and primary zones, some of which are subsequently opened, allowing extravasation.

The capillary meshwork adjoining the primary zone becomes less distinct as the secondary zone grows. By $9\frac{1}{2}$ days the latter is supplied by a uniform and purely capillary net; in thin sections its meshes inclose 2–4 decidual cells from which the large endothelial cells are separated only by an occasional reticulum fiber. Before the ninth day the cells of the secondary zone vary in form and size even within small areas. During this day they become uniform, and the tissue superficially resembles liver.

As the cylindrical implantation chamber becomes spherical during the ninth and tenth days, the implantation zone spreads out over its surface (Fig. 1, *F* and *G*). Cells degenerate here and along the surface of the implantation cavity. However, the rapid increase in the size of the cavity is the greatest factor in the thinning of the antimesometrial decidua. Owing to this pressure the cells become elongated or rectangular in form by the eleventh day, and by the twelfth day the antimesometrial decidua has been reduced to a thin decidua capsularis.

During the last of the seventh day rapid growth begins in the mesometrial region. It is due to an increase in oedema and to cellular hypertrophy. Since the growth and sinusoid formation are associated with the deposition or elaboration of glycogen, it will be considered below (cf. p. 222). The general direction of the growth may be seen in Figure 1 (*E*, *F*, *G*, and *H*) where the area is represented by hatching.

LIPOID

Typical stages of development will be considered in the description of the lipoid. Although both osmic and Sudan III preparations have been made, only the latter will be described. The antimesometrial decidua is particularly distinguished by its early accumulations of lipoid.

Five-day-22-hour stage.—The general appearance of the stroma is shown in Figure 14. Only the high columnar surface epithelial and gland cells show lipoid. Large lipoid spherules are crowded between the nucleus and the base of the cell, and a few lie just distal to the nucleus. The distal third of the cell does not contain any large spherules; however, minute rodlike granules are present and, under low magnifications, give this portion of the cell a yellowish coloration. This is true in the gland cells also in which only a few spherules are found. In a few sections of a somewhat younger specimen the decidual cells of the primary zone, which have not reached their full size, contain from 6 to 24 minute spherical lipoid granules not much larger than mitochondria. Occasionally they are all at one side of the nucleus. The cells of the secondary decidual zone also have a few small granules.

Six-day-4-hour stage.—The surface epithelium has differentiated into its four areas (cf. p. 214). The mesometrial area is the same as in the fifth-day case (Fig. 8). The transitional area shows a decrease of lipoid in the distal portion of the cell in which the basal spherules are somewhat larger (Fig. 7). The pseudostratified epithelium of the antimesometrial area has a high content of large basal spherules (Fig. 6). The flattened cells of the implantation area have relatively as much lipoid (Fig. 9).

The implantation zone is now differentiated by the fact that its cells are comfortably filled with granules two to three times the size of those in the primary decidual zone where they have increased somewhat in number and size as compared with the previous stage. In the secondary zone and in the mesometrial region the majority of the cells have a few minute granules.

Six-day-9-hour stage.—Relatively the implantation zone has not increased in lipoid content. The increase in the primary zone is due to the increase in the size of individual granules which occupy an area just around the nucleus or at one side of it. The cells adjacent to the primary zone and a few others have hypertrophied and contain a few large spherical granules in addition to the minute granules which characterize most of the secondary decidual zone. No change has occurred in the mesometrial zone.

Six-day-15-hour stage.—The implantation epithelium is gone. In most of the cells of

the transitional and mesometrial areas, the basal granules are missing, whereas the distal half of the cell still contains lipoid. In an implantation site of the same age from another animal the mesometrial and transitional epithelia are quite high and contain an abundance of basal spherules. In the lower third of the transitional area the whole cell is packed with spherules. The antimesometrial area is short and shows both pseudostratification and a heavy lipoid content.

The cells of the implantation zone are filled with large lipoid spherules which may lie separated or in groups, and often it seems as if there had been partial fusions. Under a low magnification these cells are easily mistaken for epithelial cells of the antimesometrial area. The primary decidual zone extends a considerable distance beyond the mesometrial end of the embryo (Fig. 1, *D*). Here the cells are elongated in the direction of the implantation axis. The few lipoid granules lying at one end of the nucleus approach the size of the spherules of the epithelium. At the antimesometrial end of the zone the cells have not changed.

Under low powers the lipoid in the secondary decidual zone is easily recognized. In addition to the minute granules previously found there are larger granules which may appear as spheres, crescents, or rings. They tend to be localized at one side of the cell, or they may be evenly dispersed throughout the cytoplasm. Occasionally a few fuse to form an elliptical globule. In dividing cells the granules move to the periphery and are never found in the spindle area. The binucleated cells do not differ from the rest in the content or nature of the lipoid. It is to be remembered that, although the zone is rather uniform, individual cells of any form or size, lying by themselves or in groups of two or three, may be found which are free of lipoid. Again, it is possible to find a small cell heavily loaded with granules while an adjoining cell, twice its size, may have only a few or no granules. These conditions hold true as long as the lipoid remains granular.

If a cross-section perpendicular to the implantation axis were made through the widest point of the lipoid area, the implantation zone would form a central disk heavily loaded with lipoid. Surrounding that would be found a ring poor in lipoid—the primary decidual zone. The secondary zone would form a third ring, again heavily stained with a gradual decrease toward its outer edge. The mesometrial region shows little change in lipoid content.

Seven-day stage.—The mesometrial and transitional epithelia still contain lipoid spherules, but the number is rapidly decreasing, and most of the cells of the mesometrial area show only the minute granules. Antimesometrially only a few free epithelial nuclei are found among the large, closely packed lipoid spherules. This region is quite distinct from that in which the embryonic giant cells lie. They, too, contain large lipoid spherules.

The primary decidual zone differs but little from that of the previous case. The elongate cells of the implantation zone nearest the implantation cavity take a yellowish stain, and only a few orange-colored granules are present. Here and there a cell lying free in the cavity is loaded with lipoid spherules. It is impossible to distinguish epithelial from decidual cells at this point. The remainder of the implantation zone, which is relatively narrower and shorter than in the previous case, shows no change in its lipoid content.

Practically all antimesometrial cells now contain a few large granules, and the number of cells in which they are abundant has increased. In material fixed or mordanted for 1–2 days in osmic acid mixtures the antimesometrial region is differentiated from the mesometrial in that it appears much darker. This is due to the darkening of the ground

substance of the cells, although all the granules which stain with Sudan III are not blackened. This reaction to osmic acid persists to the eleventh day, although the number of granules decreases. It should be noted that corresponding to the plasma reaction with osmic acid there is a diffuse yellowish-orange staining of the cytoplasmic ground substance in the colloidal Sudan III preparations.

The hypertrophied cells around the mesometrial end of the lumen show a number of fair-sized granules in addition to the few minute granules which are found in the remainder of the zone.

Seven-day-21-hour stage.—The entire transitional area of epithelium is now a mass of nuclei, red blood cells, and lipid granules. The mesometrial area has only the distal granules described above. However, a site from the same uterus, fixed in chilled solutions, shows in addition numerous basal spherules which are not visible in the specimen fixed at room temperature. Antimesometrially the number of nuclei has decreased. The primary decidual and implantation zones show the same lipid picture as the preceding case. This is true also of the secondary zone, except that the innermost cells usually have fewer large granules. The mesometrial region has only a few minute granules. As in the case of the epithelium, the lipid of the primary and secondary decidual zones is more abundant when chilled fixatives are used.

Eight-day-12-hour stage.—This implantation site was fixed at room temperature, and only a few of the granules have been retained. The whole of the secondary decidual zone shows the diffuse yellowish orange, or blackening, when osmic acid is used. The central half of the zone, which was richer in granular lipid in the 7-day case, now stains more intensely than the other half. All the granules have not disappeared, however. Very few cells of the mesometrial region show any indication of lipid.

Nine-day-12-hour stage.—The implantation zone has a lake of blood in its center, and the cells which surround it contain many large lipid granules. Also a few nuclei surrounded by lipid granules are found in the lake. The striking feature of the remainder of the zone is that a few lipid granules lie in the unstained cytoplasm of the cells. The primary and secondary decidual zones show a yellowish-orange coloration under low magnifications, but it is impossible to find any granules. The vacuolated cytoplasm of the mesometrial cells lying in the meshwork of sinusoids also stains with Sudan III, but granules are rare.

Eleven-day stage.—There has been no change in the lipid. The implantation zone still has granular lipid, whereas the diffuse yellowish-orange staining of the secondary decidual zone and the vacuolated cells of the mesometrial region are as pronounced as in the previous case.

Twelve-day stage.—The antimesometrial decidua, which is now a decidua capsularis (Fig. 1, H), consists only of several layers of elongated cells. They still stain diffusely. The appearance of an intermediate zone, free of lipid, is the only change found in the mesometrial region (see "Glycogen" section, pp. 222 ff.).

GLYCOGEN

Six-day stage.—Whereas in later stages the mesometrial decidua is especially characterized by intracellular glycogen, at this time glycogen is only found antimesometrially. There, it is present in approximately half of the cells of the primary decidual zone at the sides of the implantation chamber and an occasional cell beyond it (Fig. 13). It appears in the wall of vacuoles as discrete granules after fixation in formol alcohol.

The remainder of the stroma is free of glycogen. It has been impossible to demonstrate glycogen in the epithelium at any stage.

Six-day-9-hour stage.—Glycogen makes its appearance in the newly hypertrophied cells immediately adjacent to the transitional epithelium. This marks the beginning of what may be called the "glycogenic" area (Fig. 1, *C* and *D*).

Seven-day stage.—Very few cells in the primary zone stain with Best's carmine, but the glycogenic area has increased in extent.

Seven-day-8-hour stage.—Glycogen has disappeared from the antimesometrial region and does not reappear. The glycogenic area is 4-6 cells in width. Its relations may be seen in Figure 1, *D*, where it is indicated by hatching. The hypertrophied cells of this region are loaded with glycogen. The subsequent spread of the glycogenic area into the mesometrial decidua (Fig. 1, *E*) is indicated by the appearance of an occasional flake of intracellular glycogen.

Seven-day-12-hour stage.—In this case the glycogen has disappeared from the cells adjacent to the uterine lumen which accordingly resemble the cells of the primary decidual zone. They remain free of glycogen. As indicated in Figure 1, *D*, the capillaries lateral to the glycogenic area have already become prominent. In their vicinity the cells are now laying down glycogen so that the wings of the area are foreshadowed (Fig. 1, *E*).

Eight-day-5-hour stage.—In a continuation of the growth noted in the previous case, the capillaries of the glycogenic area have become sinusoidal, and some of them open into the blood space surrounding the Träger. The entire mesometrial decidua is larger. The enlarged cells, which are never binucleated, lie in groups separated by sinusoids and invested with reticulum fibers arranged in bundles. The inner edge of the glycogenic area is sharp; cells loaded with glycogen are bounded by cells free from it. Proceeding laterally the glycogenic area fades off gradually as the cells become smaller and the intracellular spaces larger. At the lateral extremities, and mesometrially, the cells are stellate, and only a few contain glycogen.

Nine-day-12-hour stage.—The sinusoids have enlarged and have become more abundant so that the glycogen-laden decidual cells appear as islands surrounded by endothelium. Their distribution is seen in Figure 1, *F* and *G*. They are the forerunners of the maternal blood channels of the definitive placenta, and at this time their distribution is largely confined to the glycogenic area. Decidual cells have been described in these sinusoids, but in most instances they are only slices of the irregular islands cut off in sectioning since they are surrounded by endothelium.

As in the earlier stage the specific characteristics of the area fade off at the periphery. With the spread of the area the mesometrial central area becomes smaller (Fig. 1, *F*, coarse hatching) as the cells at its periphery develop glycogen. Even at this stage there are scattered decidual cells in it which contain some glycogen.

Ten-day stage.—The glycogenic area with its sinusoids has pushed still farther mesometrially (Fig. 1, *G*). The decidual cells containing glycogen in the central area have increased in number but are still by far in the minority.

Eleven-day stage.—The development of the Träger and the enlargement of the gestation sac as well as the spread of the glycogenic area contribute to the reduction of the central area. The sinusoids have spread throughout the mesometrial region but are closer together in the decidua overlying the yolk sac (Fig. 1, *H*). This suggests resorption by the yolk sac.

At the narrowed antimesometrial extremity of the glycogenic area the elongated cells are vacuolated and contain much glycogen, except where they lie in contact with the embryonic tissue. Proceeding toward the implantation axis, where glycogen is much more abundant than in the previous case, typical decidual cells are encountered. The central area is now reduced in size, and fully one-half of its cells contain glycogen.

Glycogen-free endothelium lines the sinusoids where they are intact. Occasionally there is a free cell containing glycogen within them.

Twelve-day stage.—Previously the glycogenic area has been in contact with the Träger (ectoplacental cone of Duval). Now a narrow zone has appeared between them. This is due to the regressive transformation of the decidual cells which have elongate nuclei, vague boundaries, and little glycogen. Sinusoids are almost lacking.

The remainder of the maternal placenta has not changed fundamentally except for a decrease in glycogen. Another feature which may be mentioned is that in the center of the maternal placenta some of the endothelial cells have enlarged. In these sinusoids, and in the spaces of the fetal placenta containing maternal blood, there are a few free, glycogen-laden cells.

CYTOLOGICAL SUMMARY

The foregoing description clearly indicates that there are fundamental differences between the antimesometrial and the mesometrial decidual cells. First, the nucleus of the antimesometrial cell is relatively large and double in approximately one-third of the cells. One or 2 eccentrically placed nucleoli are present, and the chromatin is usually in large clumps near the periphery. The cytoplasm is decidedly granular, and a distinct cell membrane is present. Some of the granules contained in the cytoplasm may be lipoidal, and in the majority of the cells these granules fill much of the cytoplasm (Fig. 3). The more lipid these cells contain, the more difficult it is to demonstrate the mitochondria which when seen are short, fine filaments with a few long threads and spheres (Figs. 10 and 11).

The mesometrial cell does not become so large as the antimesometrial cell. Whereas the antimesometrial cell is always readily isolated in fresh material, it becomes more difficult to isolate the mesometrial cell as the gestation age increases. The nucleus differs from that of the antimesometrial cell in that it is always single and is likely to shrink in fixation. Unless the lipid and glycogen are preserved, the cytoplasm appears vacuolated. The lipid is never abundant. On the other hand, large amounts of glycogen may be present. The mitochondria differ from those of the antimesometrial cells only in that they are more readily stained.

Mesometrially there is a third type of cell the origin of which has not been determined. This compares in size to the mesometrial decidual cells, and the nucleus has an indentation at one side and stains deeply. The cytoplasm is sparse. Mitochondria are perinuclear and tend to be granular in form.

DISCUSSION

The extensive antimesometrial decidual formation is to be correlated with three conditions: first, the glands are few and do not appear so active as they are in many mammals, nutrition for the embryo being provided by the stroma; second, as Duval (1897) suggested, the reaction may be associated with the peculiar development of the fetal membranes; and, third, it provides the decidua capsularis.

The reaction has been regarded as one of growth and degeneration which ultimately leads to the formation of a capsule for the embryo by Tafani (1886), Duval (1891), Jenkinson (1902), Burckhard (1901), Kolster (1903), and others in the mouse and rat. The cytolysis of decidual cells by fetal elements has been the explanation for the enlargement of the implantation chamber. In the rat this is not entirely true. The loss of cells plays only a minor role in the thinning of the wall. When one considers that the diameter of the gestation sac at 12 days is greater than that of the entire antimesometrial decidua at 9 days, it becomes apparent that the thinning of the wall is due to the increasing amount of surface to be covered. Such tissue loss as there is can be explained on the hypothesis of physical pressure being exerted upon these cells great enough to cause their death.

The decidual reaction, then, is a normal but exceedingly vigorous growth in which the few signs of degeneration can be accounted for on a mechanical basis. Thus, the formation of binucleated cells in the antimesometrial region may be regarded as a feature of the rapid growth; they do not become multinucleated, nor is there any fusion to form a symplasma as Burckhard (1901) noted in the mouse.

It has been inferred that the presence of the lipoid in the decidual cells is to provide a source of nutritive material for the embryo. Such a concept is supported by the dispersion and increase in lability of the lipoid at the start of the eighth day. At this time the yolk sac has been differentiated and, in addition, capillaries have opened into the implantation sac. These phenomena support the suggestion of Lee (1918), Brunschwig (1927), and others that the physiological yolk-sac placentation precedes the development of a definite placenta. From this point of view the preceding period of granular lipoid development must be regarded as a storage phase.

In so far as I have been able to determine, no one has described glycogen in the antimesometrial decidua, and Szendi (1933) denies its presence in this location. There can be no question, however, that it is present in the primary decidual zone in early implantation stages. The reason for its prompt disappearance is a question that remains unanswered.

The controversy over the origin of the cells of the glycogenic area in various mammals—Maximow (1897), Chipman (1902), Goldmann (1912), Gérard (1926), and others—can be definitely settled so far as the rat is concerned. In this animal all the stroma cells of the mesometrial region may contain glycogen. Also, it is quite apparent that the formation of decidual cells is not merely a deposition of glycogen. However, the general elaboration of glycogen, as well as decidual growth, are definitely correlated with the increase and enlargement of capillaries into sinusoids. The only explanation of the decidual glycogen and lipoid is that they are food reserves for the embryo.

To summarize, the rat uterus responds to the embryonic stimulation first in the formation of an antimesometrial decidua characterized by large amounts of intracellular lipoid and then in a mesometrial reaction involving an elaboration of intracellular glycogen before the cells enlarge. It is impossible in cytological preparations to differentiate the stroma cells of the two regions. Thus, it is a question whether we are dealing with two physiologically distinct cell types, which respond differently and at different times to the same stimulus, or whether differences in blood supply or some similar condition account for the difference in response of the two regions. It will appear below that these differences are inherent in the uterine stroma and are not due to differences in the effects of embryonic structures.

THE DECIDUOMA REACTION

The deciduoma reaction, first observed by L. Loeb (1908), has been studied by various investigators, among whom may be mentioned Hammond (1917), Nielson (1921), and Brouha (1934) in the rabbit; Frank (1911), Corner and Warren (1919), and Long and Evans (1922) in the rat; Parkes (1929) in the mouse; and Krainz (1914) in the bitch. The majority of investigators have been satisfied to obtain the reaction, and it appears that none of them has been interested in the minutiae of the deciduoma. It has been shown, however, that there are various regional differentiations in normal pregnancy. The question accordingly arises: Is the deciduoma formation an unorganized tumorous reaction on the part of a sensitized stroma, or are there correlated regional differentiations comparable to those found in the normal decidual reaction? The answer is unambiguous, as will appear in the following pages.

TABLE 2*

DECIDUOMATA PRODUCED BY MECHANICAL STIMULATION

RAT NO.	GESTATION AGE AT TIME OF STIMULUS		TOTAL GESTATION		No. EMBRYOS IN NORMAL HORN	No. OF DE- CIDUO- MATA	No. OF STIMULI	NATURE OF STIMULUS
	Day	Hour	Day	Hour				
58.....	4	12	8	12	4	5	5	Cautery
62.....	2	14	6	2	5	3	5	Paraffin†
63.....	2	18	6	8	4	2	5	Thread†
64.....	3	11	6	11	3	2	5	Steel
66.....	4		6	15	0	3	7	Steel
67.....	4	13	7	23	4	5	8	Glass
69.....	4	12	7	13	4	4	6	Steel
70.....	4		7		4	5	7	Steel
72.....	4	11	6	18	4	5	5	Glass
73.....	4	11	6	21	7	6	6	Glass
76.....	4	13	9		0	6	6	Glass
81.....	4	13	12	1	4	7	9	Glass
83.....	4	12	10	15	4	4	7	Glass

* Rat No. 58 was the only multiparous animal used. In 5 cases deciduomata failed to develop when stimulation was applied before the third or after the seventh day. However, in 4 other cases the reaction failed although there were normal pregnancies in the normal horn. Of the deciduomata obtained 40 were used for study.

† Bits of thread or paraffin were inserted into the lumen through a minute incision at the tubal end of the horn.

MATERIAL AND METHODS

In order to obtain the normal hormonal balance of pregnancy the deciduomata were produced in the empty horns of unilateral pregnancies. Prior to the time of breeding, one Fallopian tube was tied and resected in part. In some cases this operation was done during pro-oestrus, but in only one case did the animal mate on the same day. The others did not mate until the next oestrus period.

In a preliminary series of experiments various methods for the production of deciduomata which have been described were tested (Table 2), and the thermocautery was also tried. However, all these methods produced trauma with some extravasation of blood. To avoid this an electrical stimulation method was devised which introduced fewer vari-

able factors and was used in a second group of animals (Table 3). The method consists of simultaneously bringing the two electrodes into contact with the mesometrial and anti-mesometrial surfaces of the empty uterine horn. There were definitely localized decidual reactions corresponding to the contact points. As many as 6 enlargements were obtained in a single horn. Twelve deciduomata thus obtained were selected for cytological study.

TABLE 3
DECIDUOMATA PRODUCED BY DIRECT-CURRENT STIMULATION

RAT No.	GESTATION AGE AT TIME OF STIMULUS		TOTAL GESTATION		No. EM- BRYOS IN NORMAL HORN	No. OF DECIDUO- MATA	No. OF STIMULA- TIONS
	Day	Hour	Day	Hour			
51.....	4	12	6	11	3	2	8
57.....	4	14	8	20	5	4	6
82.....	4	13	6	19	5	6	8
85.....	4	11	10	12	6	5	7
86.....	4	14	6	14	3	6	6
94.....	4	12	10	13	7	2	7
95.....	4	12	11	13	2	1	5

TABLE 4
DECIDUOMATA PRODUCED IN A PREGNANT HORN

RAT No.	GESTATION AGE AT TIME OF STIMULUS		TOTAL GESTATION		No. EM BRYOS IN NORMAL HORN	No. EM- BRYOS IN EXPERI- MENTAL HORN	No. OF DECIDU- OMATA	No. OF STIMU- LATIONS	NATURE OF STIMU- LUS
	Day	Hour	Day	Hour					
49*.....	4	15	9	12	2	5	2	4	Glass
53†.....	4	13	11		6	3	2	8	Glass
54†.....	4	14	9		4	2	4	4	Glass
79.....	4	13	9	22	4	2	3	4	Glass
80.....	4	13	8	13	1	0	1	6	Glass
80*.....	4	12	10		0	2	1	4	Glass
90*.....	4	12	8		7	2	4	5	Glass
91*.....	4	11	6	23	2	2	3	5	Glass

* The intact pregnant horn was stabbed.

† Successful transplants to the kidney of the removed portion of the horn.

In a third group of animals, during the fourth and early fifth days after copulation, a portion of the tubal end of the pregnant horn was removed for transplantation experiments (cf. p. 230). The remainder of the horn was then stabbed with a steel or glass needle and pregnancy allowed to continue. Fifteen deciduomata from this group were studied by cytological methods. The same methods of fixation and staining and fresh-tissue study were used as described in the first section of the paper.

OBSERVATIONS

Since the major features of development of the deciduomata so closely follow those found in implantation, it will suffice to point out the resemblances and differences between them. Also, since the electrically produced deciduomata follow the normal more closely than the others, they will be used as a basis. For convenience the period studied has been divided into daily intervals, and as nearly as possible the same features listed under the same headings. In all cases the age given represents the time elapsed since copulation so that the findings can be correlated as to time sequence with those of normal pregnancy.

SIXTH DAY

In normal implantation this is the period of embryonic attachment and the division of the antimesometrial region into its respective zones. Grossly a deciduoma may be mistaken for an implantation site of corresponding age since it is only slightly larger. When produced by mechanical stimulation, it is less sharply defined. The greater volume is due to an increase in oedema fluid in the muscular coat and in the inactive basal zone (Fig. 15). This is also true of the seventh-day cases.

Lumen.—The locus of stimulation may be regarded as analogous to the implantation axis since the growth centers about it. There is a corresponding increase in the length of the lumen in this axis, and indications of its regional differentiation are present (cf. p. 214). In the cases of mechanical injury the instrument causes an antimesometrial displacement of the lumen, and usually a tongue of tissue protrudes into it. The tongue shows signs of degeneration whether it be purely epithelial or have a core of decidual cells. Small blood clots are present in most of the mechanically stimulated cases whereas in electrical stimulation only a few leucocytes may be found.

Antimesometrial region.—The development of the primary decidual zone along the implantation axis to the basal zone, and the subsequent separation of the two zones during the last part of the day by the development of the secondary decidual zone (cf. p. 218), occur as in normal implantation. That is to say, there is hypertrophy of the primary decidual cells with some mitosis, rapid hyperplasia, a hypertrophy, and the formation of binucleated cells in the secondary zone.

Capillaries.—Although the primary decidual zone does not have many capillaries extending into it, there are a few prominent ones just under the surface epithelium antimesometrial to the lumen. They correspond in position to the implantation zone which is otherwise not differentiated.

Lipoid.—The antimesometrial cells resemble the cells of normal implantation in all essentials, including the elaboration of lipoid and its distribution in the various zones. The epithelium usually is not so high and contains fewer basal spherules, whereas the epithelial cells of the tongue which is found in the mechanical stimulation are usually loaded with lipoid.

Glycogen.—Glycogen is demonstrable in the primary decidual-zone cells. As a rule it is more abundant than in normal implantation (cf. p. 221) since it is present in most of the cells of the zone. In mechanically stimulated deciduomata glycogen does not appear until the next day. The epithelium is glycogen-free as it is in all implantation specimens.

Mesometrial region.—There is as yet no reaction here as is the case in normal pregnancy.

SEVENTH DAY

In normal implantation the granular lipoid phase reaches its climax, and the mesometrial reaction is initiated. The deciduomata show similar changes.

Antimesometrial region.—The major growth of the antimesometrium is due to the hypertrophy of the cells in the secondary decidual zone. The hyperplasia of these cells has moderated greatly as in the normal condition, and the number of binucleated cells has increased. The basal zone has decreased in extent.

Capillaries.—The capillary meshwork at the border of the primary decidual zone is loosened (cf. p. 218), and its prominence causes this stage to compare favorably with a 9–10-day normal pregnancy. By the end of the day these capillaries have begun to join the prominent capillaries along the sides of the lumen to bring about the condition found normally late in the seventh day (cf. p. 218). They are not opened, however.

Lipoid.—The deposition of granular lipoid reaches a climax in extent early in the day. At this stage a cross-section at right angles to the implantation axis would give the same picture as that of normal implantation except that the capillary bed, rather than the implantation zone, would be at the center (cf. p. 220). On the whole, the deciduomata have slightly less lipoid than the normal in that fewer cells are heavily loaded with the larger granules. However, the arrangement of granules within the cell is the same (cf. p. 220 and Figs. 4 and 5). During the later part of the day the number of granules is decreased. Occasional cells in the mesometrial region contain a few granules.

Glycogen.—The glycogen distribution is not unlike that of the implantation sites. During the course of the day it decreases in the primary decidual zone and there is a gradual migration mesometrially so that by the end of the day the glycogenic area with its associated sinusoidal meshwork is well defined. This is slightly in advance of the condition found normally.

Mesometrial region.—The mesometrial differentiation starts in the seventh day and is characterized by the differentiation and enlargement of capillaries to form sinusoids about the mesometrial end of the lumen by the end of the day. At that time they are continuous with the capillaries of the antimesometrial region, forming an incomplete vascular ring about the lumen.

Reticulum.—In fresh-tissue studies the entire decidua appears to be more fibrous than in normal implantation. This is not due to the reticulum, for in stained sections its appearance in deciduomata is very similar to that seen in the normal decidua. The only difference is to be found in the absence of the implantation zone in the former (cf. p. 218).

EIGHTH DAY

A change in the nature of the lipoid and continued mesometrial development are characteristic of normal pregnancy. In the deciduomata the following development occurs:

Lumen.—The lumen is shortened and assumes a central position during the course of the day owing to the hypertrophy of the mesometrial region. In many cases its low cuboidal epithelium is incomplete.

Antimesometrial region.—The cells in the outer portions of the secondary decidual zone continue to enlarge.

Capillaries.—The peripheral portions of the capillary meshwork of the younger stages is farther dispersed whereas the vascular ring about the lumen becomes more complete. Only the presence of the epithelium differentiates it from the normal site. At this time it fully occupies an area corresponding to that of the implantation zone, and only a few decidual cells are dispersed among the closely packed capillaries.

Lipoid.—The lipoid of the antimesometrium changes from the granular to the dispersed condition. The decrease in the number of granules is relatively the same as in normal implantation (cf. p. 221).

Glycogen.—The glycogenic area as well as the sinusoidal area develop rapidly to form the winglike arrangement noted in the normal implantation (cf. p. 222). There is a loss of glycogen in the cells bordering the lumen, and the central area of the mesometrium becomes clearly differentiated.

NINTH DAY

Uniform size and distribution of the antimesometrial decidual cells are attained, and early maternal placental formation continues as normal implantation proceeds. In the deciduomata from this time on considerable variation in growth is encountered. During the seventh and eighth days the development has been slightly in advance of implantation. At this time, particularly if a sufficient number of stabs has been made, the whole horn may be enlarged and only slight depressions in the mesometrial wall distinguish the separate areas of reaction.

Antimesometrial region.—The liver-like appearance of the antimesometrial wall (cf. p. 218) is attained by the middle of the day and is like normal implantation in all its features.

Lipoid.—The lipoid is generally dispersed, and only occasional cells contain granules. The larger mesometrial cells may have a few granules.

Glycogen.—The glycogenic area has developed in the direction described for normal implantation although it is slightly in advance of it. Thus the central area is correspondingly small (cf. p. 222).

Mesometrial region.—The cells of the mesometrial region exhibit the characters described for the corresponding normal cells and, like them, do not attain the size of the antimesometrial cells. In addition, cells with the deep-staining indented nuclei and sparse cytoplasm are also found. All three cell types are easily recognized in fresh tissue.

Sinusoids.—The distribution of the sinusoids in their relation to the lumen merits particular attention. It has been noted previously that the surface epithelium is incomplete. At this time it may be defective mesometrially, leaving areas where the endothelium borders the lumen. In places this, too, is lost, and blood is found in the lumen. In mechanically stimulated cases this does not appear to be due to the initial trauma. In the case of the electrically stimulated deciduomata the continuity of the surface epithelium was not broken at the time of the stimulation. That the sinusoids are open *intra vitam* in such cases has been shown by the intravenous injection of colloidal mercuric sulphide (Hille) into the blood stream. After such treatment blood containing the sulphide may be found in the lumen of electrically as well as mechanically stimulated deciduomata. This is strong evidence that the extravasation is a feature of early maternal placental development and is quite independent of trophoblastic activity. The blood of the placental sign of Long and Evans is then probably due entirely to maternal reactions.

The presence of leucocytes noted in the younger cases has persisted, and at this stage they are abundant in the mesometrial sinusoids.

TENTH DAY

In pregnancy the thinning of the antimesometrial wall and the continued development of the glycogenic area are the outstanding features.

Antimesometrial region.—The antimesometrial wall has increased in thickness in contrast to the normal where the implantation cavity is becoming spherical and enlarging (cf. p. 218).

Lipoid.—The lipid content does not vary from that of the normal.

Glycogen.—The glycogenic area and its associated sinusoidal net have progressed rapidly and have a structure much the same as that found in the normal 11-day case (cf. p. 222). Thus, the climax is reached approximately a day sooner, and the deciduoma has slightly more glycogen than the early maternal placenta of pregnancy.

ELEVENTH DAY

In normal pregnancy the antimesometrial wall thins to a decidua capsularis, and the glycogenic area reaches the climax of its development.

Lumen.—In the deciduoma the lumen may have appeared between the antimesometrial region and the basal zone so that two lumina appear in cross-sections of the enlargement. Both cavities are usually filled with a coagulum of blood, and cellular elements with some evidence of leucocytosis.

Antimesometrial region.—Because there is no rapidly enlarging lumen the antimesometrial wall has retained its thickness. Capillaries are abundant, and there are small areas in the central half of the region in which the cells are reduced in size.

Lipoid.—The dispersed lipid is still present.

Mesometrial region.—The sinusoids increase in number, but there is a sharp decrease in the amount of glycogen.

KIDNEY TRANSPLANTS

The portions of uterine wall which were removed from pregnant horns (cf. p. 226) were washed in normal salt solution and transplanted to the kidney. Two such transplants were successful and corresponded to 9- and 11-day pregnancies. The cells were of the antimesometrial type. Although subjected to rough treatment in transplanting, only localized developments occurred. Not only were some of the cells binucleated but they also showed the condition of the lipid corresponding to the respective stage of development. The inactive portions were comparatively free of lipid. The surface epithelium, however, showed the typical pregnancy picture (cf. p. 215).

DISCUSSION

From the foregoing it is obvious that the growth of the deciduoma closely simulates the normal development of the decidua. This is especially true of those produced by electrical stimulation. The general histological features—that is, cellular distribution, time sequence, and vascular pattern, including extravasation—follow normal implantation closely. Cytologically the cells are fundamentally the same in nuclear and cytoplasmic structure and in the stored lipid and glycogen.

Mechanical stimulation introduces certain insignificant complications such as the displacement of the lumen and its protruding tongue of tissue. The major differences between deciduomata and normal implantation sites are: (1) the absence of a rapidly enlarging implantation cavity; (2) associated with this, a distinct implantation zone does not develop although it is indicated by capillary differentiation; (3) mesometrially the central area is usually smaller than in pregnancy; (4) the major growth and glycogen distribution reach their climax during the tenth day whereas it is not reached in the normal until the eleventh; and (5) the greater part of the surface epithelium is not lost.

The explanation of how the epithelium is removed and the decidual reaction initiated by the embryo has not, as yet, been found. Turner (1876) suggested that the embryo acted as a parasite and as such removed the surface epithelium and initiated the decidual reaction. The majority of the morphologists have accepted this theory in the interpretation of their observations. Selenka (1883) gave such an interpretation to the reaction in the mouse and was followed by Spee (1901) for the guinea pig, Lee (1903) for *Citellus*, and various other authors who have studied rodents.

This interpretation, however, is not conclusive. Hubrecht (1890), although he left the impression that the embryo destroyed the surface epithelium, was not certain of this fact. Sobotta (1903) in a footnote suggests that the epithelium is removed as a result of physical pressure. Ercolani (1879) had observed that there was a proliferation of the stroma tissue prior to the time of embryonic attachment. Hubrecht (1890) confirmed this phenomenon in the hedgehog and agreed with Ercolani in recognizing that such a growth was normal in many mammals.

Specifically, for the rat, considerable evidence militates against the theory of active invasion by the embryo:

1. Momentary electrical stimulation involving no mechanical injury produces the development of a decidua which histologically and cytologically closely simulates the series of changes seen in normal pregnancy. These observations make it quite clear that this development is to be regarded as a definite and organized growth of specific regions in the fulfilment of their normal physiological function and lead us to question Loeb's (1908) suggestion that the deciduoma is essentially a tumorous growth.

2. In addition to the general histological and cytological development there is a corresponding vascular differentiation which proceeds as in pregnancy even to an extravasation of blood. Our observations on this point substantiate and elucidate the findings of Evans (1928). In vitamin-E deficient rats he not only observed spontaneous deciduoma development but also the placental sign (placental bleeding) in pseudopregnant animals. More striking is his observation of the same phenomena in 4 per cent of normal pseudopregnant animals. It is evident not only that the cells of the uterus elaborate food reserves for the embryo but also that maternal blood is provided even though the embryo is not present.

3. The majority of the deciduomata described by previous workers have been produced in pseudopregnant animals. Since we have also obtained normal deciduoma development of $9\frac{1}{2}$ days in animals in which implantation in the normal horn had failed, we can rule out any possible hormonal influence of the embryo in the differentiation of the decidua.

4. The observation of Brouha (1928) and others, that the deciduoma reaction will not occur unless the stimulus is applied within certain time limits, has been confirmed, i.e., under conditions of proper hormonal balance.

Thus, it is evident that the embryo is not essential for the development of the decidual reaction and that the stimulus need not be specific. The exact nature of the stimulus normally provided by the embryo must be left to future investigation.

Furthermore, once the reaction is started, what are the factors which direct and regulate its development? A partial answer to this question is found in the appearance of a deciduoma reaction in transplanted uterine stroma. The normal location, vascularization, and innervation are not necessary as has been shown by the transplantation experiments of L. Loeb (1908) and our own (cf. p. 230). From the evidence thus far obtained it

appears that we are dealing with an organ of specific potencies and that the inherent nature of the sensitized uterus merely requires some slight stimulus, or irritation, to initiate activity which continues for at least the first half of pregnancy when the proper hormonal environment is provided.

SUMMARY

1. In normal pregnancy the decidual response of the mucous membrane is different in the mesometrial and antimesometrial regions: (a) It appears first antimesometrially on the sixth day after insemination. (b) Here the stroma cells undergo hypertrophy and hyperplasia and accumulate labile intracellular lipid. Binucleated cells are common and characteristic of this region. (c) The mesometrial reaction appears on the eighth day. The enlarged cells are loaded with glycogen, and the definitely localized dilation in the capillary bed is followed by some extravasation of blood.

2. Deciduomata were produced in most instances in the sterile horns of unilateral pregnancies. By electrical stimulation under conditions of proper hormonal balance deciduomata are produced which closely resemble the normal decidua, both in general form and in the vascular patterns. Extravasation occurs at a corresponding stage although no vessels were traumatized at the time of stimulation as they are by the methods of mechanical stimulation used hitherto. Both mechanical and electrical stimulation result in the development of characteristic antimesometrial and mesometrial decidual cells which elaborate lipid and glycogen in the typical fashion.

3. Antimesometrial deciduae with characteristic cells were obtained in bits of uterus transplanted to the kidney. This indicates that the specific reactions are inherent in the stroma cells and are not due to their position and relations in the uterus.

Since the completion of this manuscript the paper of Selye and McKeown (1935) has come to my attention. Although the paper is devoted chiefly to a consideration of the hormones involved in placental formation and the structure of the "metrial gland," there are certain features bearing on our problem. On a histological basis these authors substantiate our findings (1935) of a differentiation of antimesometrial and mesometrial regions in deciduoma development. Their conclusion that the maternal blood vessels are opened without the activity of fetal ferments is fundamentally correct, but no adequate evidence for it is presented. After electrical stimulation and good fixation the blood sinus Selye and McKeown speak of proves to be the uterine lumen. In our material the sub-epithelial region (cf p. 229) is filled with sinusoidal vessels whose endothelium is intact, and the extravasation of blood occurs only in those regions in which the surface epithelium of the lumen is lacking; there is no indication of extravasation into the decidual tissue.

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PLATE I

Camera lucida drawings of cells fixed in Zenker-Bensley, imbedded in gelatin, cut after freezing, and stained for lipoid with Sudan III. $\times 1440$.

FIG. 2.—Decidual cells of the primary decidual zone of a 6-day-4-hour pregnancy.

FIG. 3.—Cells of the secondary decidual zone of a 7-day pregnancy.

FIG. 4.—Cells of a secondary decidual zone of a deciduoma corresponding to a 7-day pregnancy.

FIG. 5.—Cells from the secondary decidual zone of a deciduoma corresponding to an 8-day pregnancy.

FIG. 6.—Epithelial cells of the antimesometrial area of a 6-day-4-hour pregnancy. These cells, as well as those shown in Figures 7, 8, and 9, are all from the same section.

FIG. 7.—Epithelium of the transitional area showing in part the transition to the implantation area.

FIG. 8.—Mesometrial epithelium.

FIG. 9.—Implantation epithelium.

PLATE II

Camera lucida drawings showing mitochondria of the antimesometrial cells. Fixed in Zenker-Bensley and stained with aniline acid fuchsin followed by Mallory's connective-tissue stain. Four μ thick. $\times 1440$.

FIG. 10.—Cells of the secondary decidual zone of a 6-day-15-hour stage.

FIG. 11.—Cells of the primary decidual zone of a 6-day-2-hour stage (gelatin-imbedding and frozen-section technique).

PLATE III

FIG. 12.—A section of mesometrial decidua of an 8-day pregnancy. The cells bordering the lumen are glycogen-free as are the endothelial cells of the sinusoids. Fixed in formol-absolute alcohol and stained with Best's carmine. Initial $\times 115$.

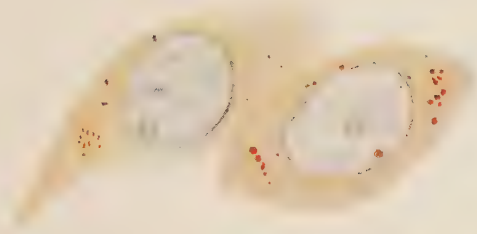
FIG. 13.—A cross-section of a 6-day implantation site. Only the implantation chamber with the surrounding primary decidual zone is shown. The differentiation in the surface epithelium is apparent. The cells of the primary decidual zone are to be regarded as decidual cells with a number of them showing glycogen in their cytoplasm. Fixed in formol-absolute alcohol and stained with Best's carmine. Initial $\times 65$.

FIG. 14.—Cross-section through the implantation site of a 5-day-22-hour pregnancy. The distribution of the glands indicates the differentiation of a mesometrial and antimesometrial region. Furthermore, the compactness of the predecidual cells about the antimesometrial two-thirds of the lumen is the first indication of the primary decidual zone. Lipoid is only present in the epithelium of the lumen and the glands. Fixed in Zenker-Bensley and stained with Sudan III. $\times 25$.

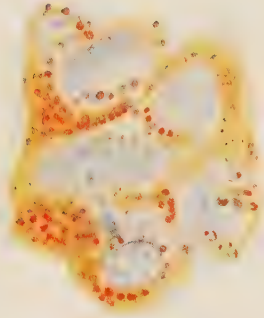
PLATE IV

FIG. 15.—Cross-section of a deciduoma corresponding to a 6-day-21-hour pregnancy. The larger size of the growth, when compared to the normal, is seen to be due to the oedematous condition of the basal zone. The lumen has been pushed mesometrially, and the lighter region extending antimesometrially from it is the primary decidual zone. Note that but few capillaries extend into it although they are numerous in the lipoid-laden secondary decidual zone. There is a slight reaction of the mesometrial region. Fixed in Zenker-Bensley and stained with Sudan III. Initial $\times 20$.

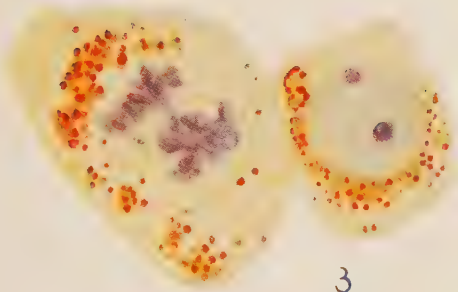
FIG. 16.—A cross-section of a deciduoma corresponding to a 9-day-12-hour pregnancy. The section passes through the deciduoma slightly to one side of the center of reaction; thus the central area of the mesometrial region is not shown. However, the cells bordering the mesometrial surface of the lumen are glycogen-free. The quantity of glycogen is comparable to that of an 11-day pregnancy as are the development and distribution of the sinusoids. The antimesometrial decidua is seen to be quite uniform. No myometrium is included. Fixed in formol-absolute alcohol and stained with Best's carmine. $\times 25$.



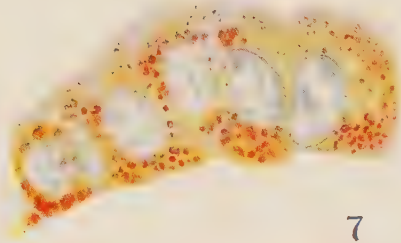
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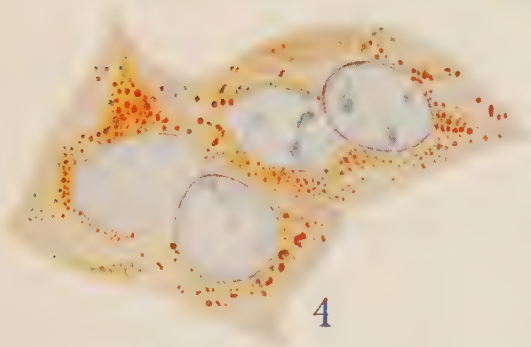
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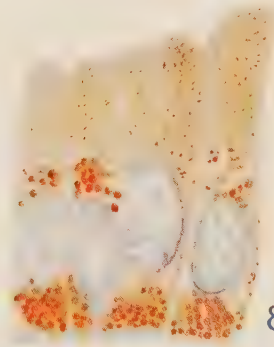
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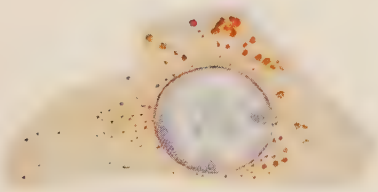
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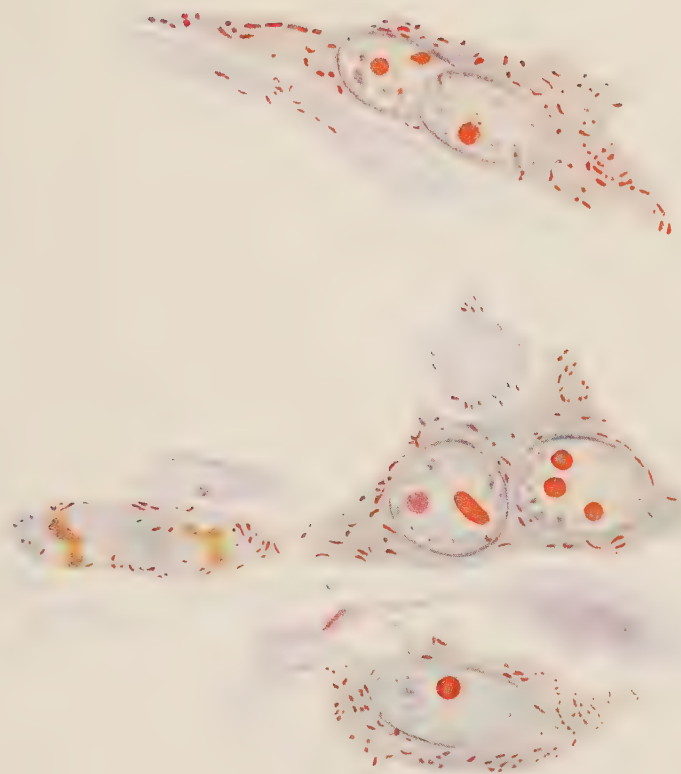


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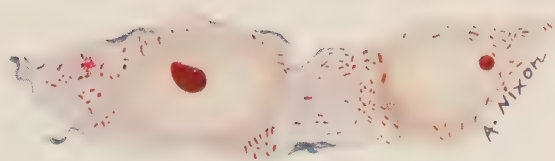


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A. Nixon

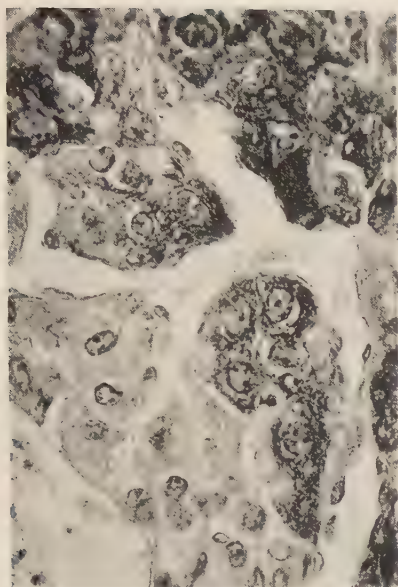


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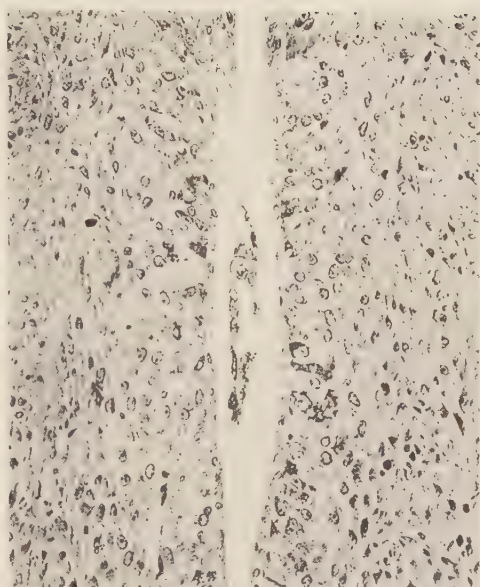


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PLATE III



12

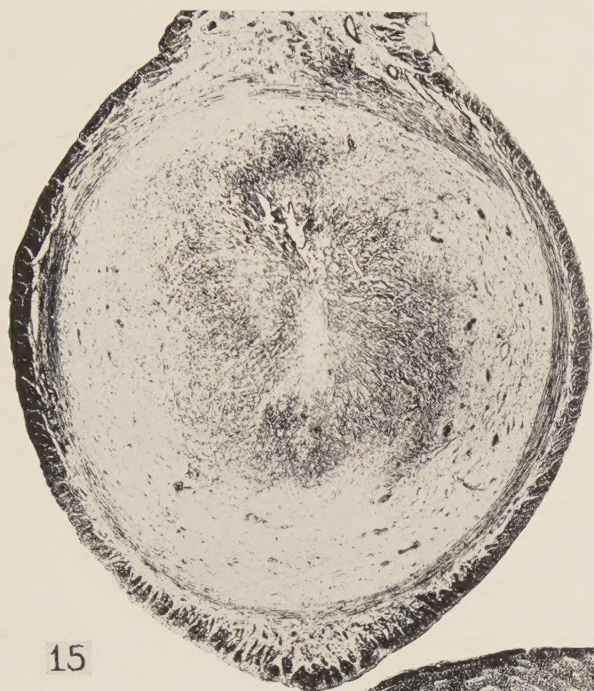


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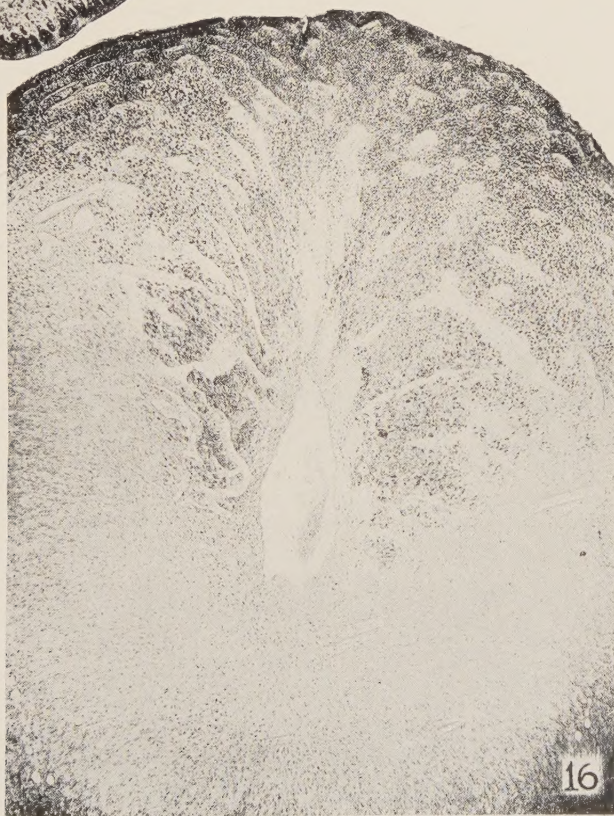


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PLATE IV



15



16

